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Intracerebroventricular Infusion of Nerve Growth Factor in Three Patients with Alzheimer's Disease

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Abstract

Nerve growth factor (NGF) is important for the survival and maintenance of central cholinergic neurons, a signalling system impaired in Alzheimer's disease. We have treated 3 patients with Alzheimer's disease with a total of 6.6 mg NGF administered continuously into the lateral cerebral ventricle for 3 months in the first 2 patients and a total of 0.55 mg for 3 shorter periods in the third patient. The patients were extensively evaluated with clinical, neuropsychological, neurophysiological and neuroradiological techniques. Three months after the NGF treatment ended, a significant increase in nicotine binding was found in several brain areas in the first 2 patients and in the hippocampus in the third patient as studied by positron emission tomography. A clear cognitive amelioration could not be demonstrated, although a few neuropsychology tests showed slight improvements. The amount of slow-wave cortical activity as studied by electroencephalography was reduced in the first 2 patients. Two negative side effects occurred with NGF treatment: first, a dull, constant back pain was observed in all 3 patients, which in 1 patient was aggravated by axial loading resulting in sharp, shooting pain of short duration. When stopping the NGF infusion, the pain disappeared within a couple of days. Reducing the dose of NGF lessened the pain. Secondly, a marked weight reduction during the infusion with a clear weight gain after ending the infusion was seen in the first 2 patients. We conclude from this limited trial that, while long-term intracerebroventricular NGF administration may cause certain potentially beneficial effects, the intraventricular route of administration is also associated with negative side effects that appear to outweigh the positive effects of the present protocol. Alternative routes of administration, and/or lower doses of NGF, perhaps combined with low doses of other neurotrophic factors, may shift this balance in favor of positive effects.

Key Words

Nerve growth factor
Alzheimer's disease
Cognitive deficits
Electroencephalography
Positron emission tomography
Pain
Weight loss
Nicotinic receptors

Introduction

Nerve growth factor (NGF) was the first neurotrophic factor to be discovered [1]. This neurotrophin supports survival and growth of fibers from specific sets of both peripheral and central neurons [1]. The extensive animal research background on NGF and its specific effects on subsets of neurons made it the first growth factor chosen for clinical trials to support intraputaminal adrenal medullary autografts in Parkinsons's disease [2, 3]. It is

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believed that the cholinergic projections from the basal forebrain to the hippocampus and cortex cerebri are important for cognition and memory. These projections undergo severe degenerative changes in Alzheimer's disease. The cholinergic neurons in the basal forebrain have been shown to be NGF sensitive [4]. In patients who suffer from Alzheimer's disease, cholinergic neurons still express NGF receptors [5]. Extensive animal work indicates that cholinergic lesions leading to cognitive disturbances in animals can be counteracted by NGF treatment [6, 7]. For a review of NGF as a possible treatment of Alzheimer's disease, see Olson [8]. Importantly, targeting the NGF gene leads to cholinergic deficits. Interestingly, mice heterozygous for an NGF mutation show cholinergic deficits late in life, suggesting that NGF dosage correlates with cholinergic phenotype [9].

The rationale behind infusion of NGF into the cerebrospinal fluid (CSF) of the lateral brain ventricle is based on the wish to reach not only areas with cholinergic nerve cell bodies but, more importantly, the vast cholinergic termination areas. CSF is not only produced by the choroid plexus, but also in brain tissue. The part of CSF which reaches the subarachnoid space moves along the perivascular channels into the cerebral cortex where it is absorbed [10]. This observation suggests that NGF administered directly into the CSF should be able to reach deeper cortical sites.

Based on the previous experience with chronic infusion of NGF into the brains of parkinsonian patients, and the knowledge that Alzheimer's disease is progressive and at present not curable, a small pilot study was initiated with infusion of NGF into the cerebral lateral ventricle of 3 patients with Alzheimer's disease. The prime aim was to investigate whether the cognitive deficits could be improved by the addition of exogenous NGF. The results from the first patient have been reported [11]. In the following, we present data from the additional 2 patients and summarize the results from the 3 patients.

Materials and Methods

Patients

The diagnosis of Alzheimer's disease in the 3 patients was based on DSM-III-R [12] and NINCDS-ADRDA criteria [13]. The patients were extensively evaluated clinically, tested with a cognitive test battery, electroencephalography (EEG), magnetic resonance imaging as well as positron emission tomography (PET) before inclusion in the study. Confounding somatic diseases or psychiatric disorders were part of the exclusion criteria. The patients, as well as their spouses, received written and oral information about the study and gave their consent. The study was approved by the Human Ethics Committee of the Karolinska Institute. In 2 of the patients, a definite diagnosis of Alzheimer's disease was obtained by neuropathological evaluation of brain biopsies (diameter of 2 mm) secured when neurosurgically inserting the catheter. All 3 patients had histories of short periods of low back pain that were alleviated with mild analgesics and had never warranted professional treatment or examination. Patient 1 is a former lawyer, who worked until retirement at the age of 65 years. No heredity for Alzheimer's disease is known. Early symptoms of cognitive deficits at the age of 61 years were observed by her husband. At the time of treatment, the Mini Mental State Examination (MMSE) [14] score had decreased to 18. She was no longer able to live at home and is presently in a nursing home. Patient 2 is a former teacher, who due to her illness was unable to continue working. Her mother had a history of cerebrovascular disease with concomitant memory decline. No heredity for Alzheimer's disease is known. At the age of 54 years, the patient started to display memory disturbances and dyspraxia. She was diagnosed as suffering from Alzheimer's disease in 1990. Before treatment the MMSE score was 23. She lived at home during treatment but is now in a nursing home. Patient 3 is a former administrative employee, whose cognitive decline was first noted in about 1990 at the age of 56 years. The patient was referred to the investigators in 1992. There is no history of Alzheimer's disease in the family. Before treatment the MMSE score was 23. He lived at home during treatment with a part-time personal assistant as aide. He is currently in a nursing home after suffering from a stroke in December 1996.

NGF Delivery System and Neurosurgery

In order to secure uninterrupted delivery of NGF in patients with limited compliance, we used completely implantable, remote-controlled infusion systems that could be refilled transcutaneously [11]. Under general anesthesia, a Silastic catheter with multiple holes in its distal part was stereotactically implanted into the right cerebral lateral ventricle as described previously [11]. Two cortical, cylindrical biopsies for neuropathological evaluation were taken from tissue which would have been otherwise destroyed by the cannula insertion. A programmable pump (Synchromed, Meditronic) was implanted subcutaneously in the lower right abdominal wall and connected to a catheter tunnelled subcutaneously in continuity with the intraventricular catheter. In the first patient, the NGF infusion was started immediately after the operation, while it was delayed 2 and 4 weeks postoperatively in patients 2 and 3, respectively. A total of 6.6 mg of NGF was infused continuously for 3 months in the first 2 patients. In the third patient, 0.21 mg of NGF was infused for 2 weeks, then the infusion was stopped and after 5 weeks NGF was restarted with a total of 0.24 mg of NGF for a period of 10 weeks. A third treatment period was started 8 months later with a total NGF dose of 0.11 mg over 10 weeks (table 1).

Nerve Growth Factor

β-NGF purified from male mouse submandibular glands [15] was used. Concentration of NGF and doses administered are shown in table 1. Sterilization procedures have been described elsewhere [11]. NGF bioactivity was assayed using a fiber outgrowth system with explanted sympathetic ganglia from chick embryos [15].

Measurements of NGF and Antibodies to NGF

CSF samples were obtained by lumbar puncture performed between 9 a.m. and 1 p.m. before, during and after the treatment period. Samples were frozen (–70°C) and later analyzed for NGF,

Table 1. Patient data

Patient characteristics	Patient 1	Patient 2	Patient 3
Age at onset of treatment, years	69	57	61
Sex	female	female	male
Time since onset of Alzheimer's disease, years	8	3	4
Time of NGF treatment	3 months	3 months	2 weeks (period 1) 10 weeks (period 2) 10 weeks (period 3)
Dose of NGF, μg/24 h	75 (6.6)	75 (6.6)	16 (0.21) (period 1) 3.4 (0.24) (period 2) 1.6 (0.11) (period 3)
Concentration of NGF infused, μg/ml	208	208	67
Volume of NGF infused, μl/24 h	360	360	240 (period 1) 50 (period 2) 24 (period 3)

Figures in parentheses indicate total dose, in milligrams.

NGF antibodies and substance P as well as routine analyses to check for signs of infection and protein leakage. Blood samples for analysis of NGF and its antibodies were also collected together with the CSF samples. NGF levels in blood and CSF were measured using an immunoassay [16]. The blood samples were tested for the possible development of anti-NGF antibodies using a specific immunoassay [11].

Radioimmunoassay for Substance P

Samples of CSF were purified on reverse-phase cartridges as described earlier [17]. Substance P was analyzed using antiserum SP2 [18] which reacts with substance P and substance P sulfoxide but not with other tachykinins. The detection limit was 10 pmol/l. Intra- and interassay coefficients of variation were 7 and 11%, respectively [18].

Clinical Evaluation

The patients were evaluated regularly including careful clinical examination and careful monitoring of potential side effects as well as routine blood sampling. Since pain developed as a negative side effect, we attempted to assess the pain by using the visual analogue scale where the patient estimates the intensity of his pain ranging from 0 (no pain) to 100 (maximum pain). However, only the third patient was able to use it. The cognitive deficiences in patients 1 and 2 prevented them from using the visual analogue scale for pain assessment.

Cognitive Testing

The cognitive evaluation was mainly directed at measuring different aspects of memory. The subjects were evaluated before treatment, 1 month after treatment had been initiated and after treatment had been stopped. Two baseline testings (3 weeks to 2 days) before the start of treatment were performed. Patient 3 could not be tested at all during his first period of NGF treatment, due to negative side effects, but was able to be tested with the lower NGF dose during treatment period 2. To assess the general level of cognitive functioning, the MMSE [14] was used. Tests for visual, spatial and verbal episodic long-term memory as well as verbal short-term memory were presented. Some aspects of attention and executive function were also evaluated. The following tests were included in the test battery: *list of 12 words,* immediate free recall and recognition for words presented visually and auditively [19]; *selective reminding,* list learning of 10 words on 5 consecutive trials with selective reminding [20]; *face recognition,* recognition of 12 individually presented faces among 12 distractors [21]; *spatial memory,* memory for placement of 20 objects [22]; *digit span,* span for digits forward and backward [23]; *verbal fluency,* fluency in one minute's time for words beginning with the letters F, A and S [24]; *trail making tests A and B,* speed tests for connecting digits and connecting both digits and letters alternating between the two systems [25]. For recognition of the 12-word list and faces as well as for placement of objects, a delayed recall after 30 min was requested. In order to minimize progressive error effects [26] different, but equivalent, versions of all tests were given at each measurement occasion.

EEG Recordings

Multiple recording sites were used for standardized EEG analysis and computer-assisted power spectrum recordings as earlier described [11] before, during and after treatment.

Positron Emission Tomography

The PET studies involved a multitracer system consisting of (S)(–)-[N-^{11}C-methyl]nicotine (cholinergic nicotinic receptors), $H_2{}^{15}O$ or ^{11}C-butanol (blood flow) [27] and [^{18}F]fluoro-2-deoxy-*D*-glucose (glucose metabolism). The PET studies were performed sequentially in the order $H_2{}^{15}O$, (S)(–)-^{11}C-nicotine and ^{18}F-fluorodeoxyglucose. A venous catheter was inserted into the right cubital vein for injection of radioactive compounds. A catheter was also inserted into the left radial artery to provide arterial blood samples to be monitored by a radiation detector. The head of the patient was positioned in the tomograph so that the lowest horizontal section included the cerebellum. The uptake and time course of (S)(–)-[N-^{11}C-methyl]-nicotine in the brain were monitored by PET following intravenous injection of a tracer dose [28]. ^{11}C-nicotine (up to 70 μg

nicotine) was given as a bolus dose over a 5- to 10-second interval, during which time the PET camera (PC 2048, PC 4096 GEMS AB Scanditronix, Uppsala, Sweden) was started. Arterial blood samples were continuously drawn by a syringe pump for 5 min to obtain the tracer concentration in blood with a resolution of 1 s. Another 5–7 blood samples were taken during the rest of the PET investigation. PET images were continuously recorded with frame lengths increasing from 12 to 600 s for predetermined periods of 25 min and thereafter reconstructed. The size of each region of interest for tracer kinetic evaluation varied between 1.2 and 40 cm^3. The radioactivity values obtained were corrected for physical decay up to the time of injection. The relative uptake and distribution of radioactivity was calculated from the corrected radioactivity per cubic centimeter and divided by the radioactive dose per gram body weight. A two-compartment model was used to analyze the ^{11}C-nicotine data [29]. In this model, K_1 is the rate constant for passage of the ligand from blood to brain tissue and K_2 is the net rate constant for the passage of the ligand from brain tissue to blood. In order to eliminate the influence of cerebral blood flow rate on the K_2 constant, it is divided by the corresponding K_2 value for $H_2{}^{15}O$ (cerebral blood flow). The resulting flow-compensated parameter k_2^* (K_2 nicotine/K_2 H_2O) [29] is independent of blood flow and reflects tissue binding of nicotine. A decrease in the k_2^* value indicates an increased binding of ^{11}C-nicotine in the brain [29]. The brain glucose metabolism was monitored by ^{18}F-fluorodeoxyglucose [30]. The patients were evaluated with the PET technique before treatment, 1 month after the treatment had been started and 3–7 months after the treatment had been stopped.

Results

The patients tolerated the surgical procedure with implantation of the infusion system reasonably well except for a short period of confusion postoperatively. Therefore, in patients 2 and 3 the start of the NGF infusion was postponed 2–4 weeks postoperatively to give the patients ample time to recover from the surgical procedure.

Using a bioassay it was demonstrated that the NGF used was fully biologically active. As expected, NGF was only present in the CSF during the infusion, and no traces of the substance could be detected in serum or CSF before or after the infusion periods. There were no anti-NGF antibodies in the CSF during or after treatment. No antibodies to NGF could be detected in peripheral blood during or after the NGF infusion.

The first 2 patients received 75 μg of NGF/24 h for 3 months. The CSF concentration of NGF during treatment was approximately 200 ng/ml. Due to negative side effects (see below), the dose of NGF given to the third patient was reduced. The third patient thus received 16 μg NGF/24 h for 2 weeks yielding an NGF concentration in CSF of 50 ng/ml. Later, he was given 3.2 μg NGF/24 h for 10 weeks (CSF 10 ng/ml) and finally 1.6 μg NGF/24 h for another 10 weeks (table 1).

Cognitive Function

The patients were tested twice before treatment was started and a variability in the test results was found. During the NGF infusion the patients suffered from side effects (see below), which made the testing difficult and may have interfered with the results. The planned follow-up testing at regular time intervals could not be carried out and a more individual testing scheme was adopted.

The MMSE scores did not improve or stabilize with treatment in any of the patients. Instead a decline over time was seen (fig. 1). Especially in patient 1, a marked reduction in MMSE scores during treatment was observed, probably partly due to side effects (see below). The overall cognitive assessment did not show any clear effect of NGF after 4 weeks of treatment compared to the baseline measurements. Patient 1, however, did show a slight improvement in the selective reminding test and delayed recognition of word list. Patient 2 showed a slight improvement in delayed recognition of faces and word list. Patient 3 did not show any improvement in any subtest (table 2). Patient 1 was again tested 10 weeks after the NGF infusion had been stopped, and the improved results in selective reminding and delayed recall of word list found during treatment were no longer seen. The immediate recognition of faces improved somewhat as compared to the test level at 4 weeks after treatment had been started. Patient 2 could not be tested with the initial test battery after the NGF infusion had been stopped. Some elementary tasks were tested on 5 occasions between 1 week and 3 months after infusion and a slight improvement was found, but the patient did not improve her cognitive functions enough to be tested with the previously used tests. Patient 3 was tested right after the third NGF infusion period of 1.6 μg/day had been ended (13 months after the start of the initial NGF treatment). The delayed recognition of faces showed a slight improvement, while the other tests showed a further decline as compared to the results before the NGF infusions started.

Imaging

In patient 1, a marked increase in the cerebral blood flow in the frontal and temporal cortices that outlasted the NGF treatment by several months was observed. The other 2 patients did not show such an improved cerebral blood flow.

A general improvement of the glucose metabolism was observed in patient 2 after the end of the NGF treatment. No marked effect on glucose metabolism was observed in patient 3. However, the glucose metabolism in cortical

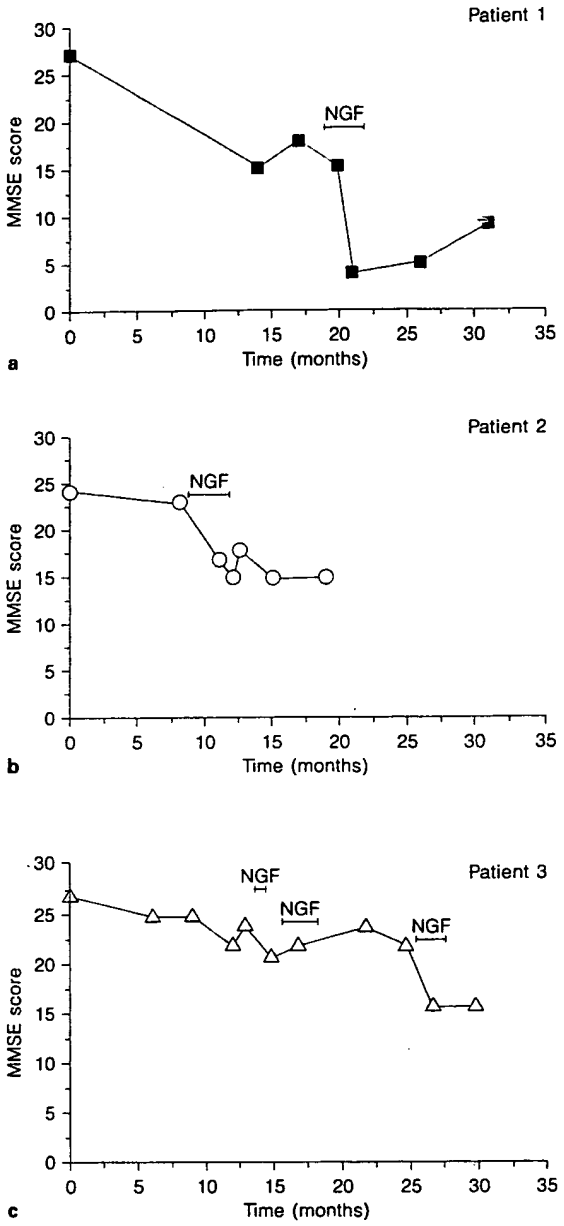

Fig. 1. MMSE scores (maximum score is 30) before, during and after treatment in each patient (**a–c**). There is a continuous decline over time. Especially in patient 1, there is a marked reduction in MMSE scores during treatment.

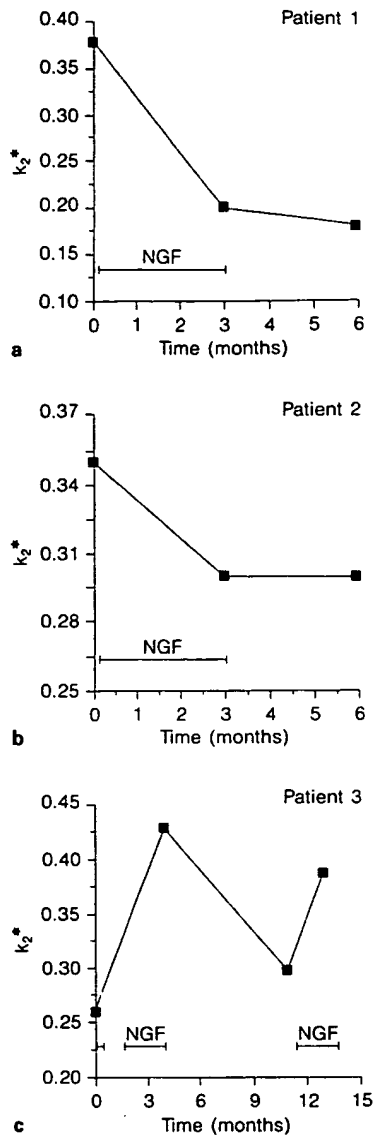

Fig. 2. The effect of NGF treatment on ^{11}C-nicotine binding (expressed as k_2^* values) in the frontal cortices of the 3 Alzheimer patients. A decrease in k_2^* values (increased ^{11}C-nicotine binding) was observed in patients 1 and 2 after 3 months of NGF treatment, as well as 3 months after infusion (**a** and **b**). **c** In patient 3, directly after the end of the second period of NGF treatment, an increase in the k_2^* value (decreased ^{11}C-nicotine binding) was found. Seven months later, prior to the start of the third NGF treatment period, the k_2^* value was decreased but was again increased after the third treatment period.

brain regions remained fairly constant during the whole period of NGF treatments compared to the decrease in glucose metabolism that had been observed during the year prior to the initiation of the NGF treatment.

When the binding of ^{11}C-nicotine in the brain was expressed as the kinetic constant k_2^*, a decrease in k_2^*

Table 2. Cognitive performance in tests of episodic memory before treatment with NGF (the mean from the two testing occasions is presented) and after 4 weeks of treatment

Test variable	Scores					
	patient 1		patient 2		patient 3	
	before	4 weeks	before	4 weeks	before	4 weeks
Face recognition						
Immediate	1.26	-0.51	1.87	0.68	1.9	1.39
Delayed	1.74	0.88	1.24	1.60	1.27	0.68
Word recall	0	0	4	2	2	2
Word recognition						
Immediate	1.03	-0.24	2.85	1.00	2.28	1.62
Delayed	0.51	1.12	0.88	1.15	1.55	0.88
Selective reminding						
Total recall	2	6	5	4	11	11

Face and word recognition data are presented as d'values. Maximum scores are: immediate and delayed face and word recognition = 4.64; word recall = 12; selective reminding = 40.

(increased binding) was observed in the frontal and temporal cortices of patient 1 after 3 months of NGF treatment and also at 3 months after infusion as compared to the preinfusion value. Similarly, a decrease in the k_2* value was observed in the frontal, temporal and parietal cortices as well as in the hippocampus in patient 2 after 3 months of NGF treatment and also 3 months after the end of treatment as compared to the value before treatment. The k_2* values were generally increased (decreased binding) in the frontal, temporal and parietal cortices in patient 3 during and after the end of the different periods of NGF treatments. A decrease in the k_2* value (increased binding) was however observed in the hippocampus of patient 3 seven months after the end of the second period of NGF treatment. When patient 3 was investigated by PET after the third NGF treatment period (13 months after starting the initial NGF treatment), k_2* was found to be increased above the baseline value. The effect of NGF treatment on the k_2* values is illustrated in figure 2 for the frontal cortex of the 3 patients.

Neurophysiological Evaluation

In patients 1 and 2 there was a reduction in slow-wave activity expressed as an increased alpha/theta ratio during treatment (fig. 3). In patient 3 there were no significant changes in EEG activity before, during or after the three treatment periods.

Side Effects

Two types of complications were noted, which may be directly linked to the NGF treatment. The most prominent side effect, observed in all 3 patients, was the development of pain. The first patient complained of muscle pain in the lower back that appeared 11–14 days after the start of NGF infusion. The pain was alleviated using dextropropoxyphene. Eleven days after the start of the infusion, the second patient reported a dull, constant muscle pain in the lower back, in the neck region and the lower limbs which was aggravated by ambulation. Peripherally acting analgesics had little effect. When the NGF infusion was stopped, the pain disappeared within 2 days. In the third patient, the dose of NGF was reduced to 16 μg/24 h resulting in a concentration of NGF in the CSF of 50 ng/ml (table 1). Four days after receiving this dose of NGF, the patient experienced constant muscle pain in his lumbar region, but there was no muscle tenderness. The pain was similar to his former low back pain, but the intensity was much higher. The pain was worsened by axial movements such as jumps, making it very difficult for the patient to travel by car. He estimated the pain to 90 on the visual analogue scale. The NGF infusion was stopped after 2 weeks, and the pain disappeared in 2 days. The infusion was again started 5 weeks later, but now the dose was further reduced to 3.4 μg/24 h. The resulting concentration of NGF in the spinal fluid was then 10 ng/ml. After 4 days of treatment the pain returned, still provoked by axial movements, but was reduced in intensity and estimated to 60. Dextropropoxyphene alleviated the pain to about 10 on the visual analogue scale. Diclofenac, paracetamol or salicylic acid did not relieve the pain. When the NGF infusion was stopped after 2 months, the pain caused by axial movements disappeared completely as before, but the dull back pain intermittently returned, now with a lower intensity than during the NGF treat-

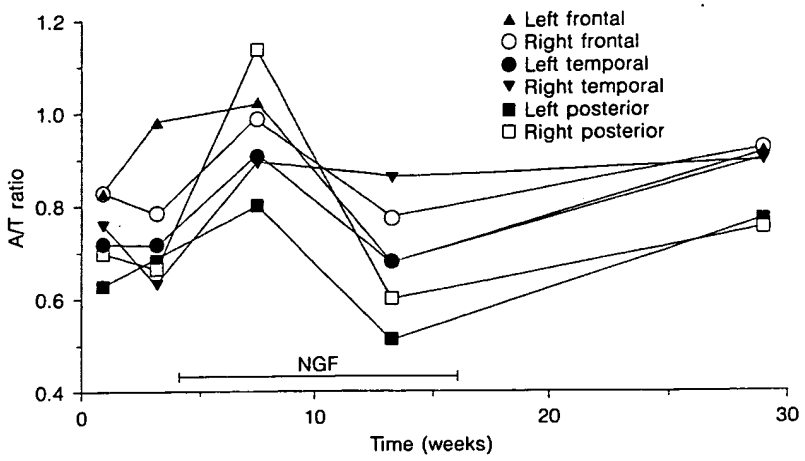

Fig. 3. The alpha/theta (A/T) ratio, obtained from EEG recordings in patient 2, increased initially during treatment with NGF, reflecting a reduced slow-wave activity. However, this improvement was not seen during the last month of treatment.

ment. The family and the patient found a subjective improvement in the cognitive functions during and after the periods of NGF infusion. In spite of the pain, which was judged tolerable at the lower NGF dose, the patient expressed a wish for an additional treatment period. After careful considerations it was decided together with the patient and his family to treat him again using a further reduction of the NGF dose. Eight months after the previous treatment period, NGF was thus infused for ten weeks in a dose of 1.6 μg/24 h. The pain caused by axial movements returned, but it was of low intensity and could be controlled with dextropropoxyphene. The pain disappeared completely when terminating the infusion. There were no clinical signs of inflammation in any of the 3 patients to explain the development of pain. Substance P in CSF was measured before, during and after treatment in all 3 patients, but no differences in substance P concentrations were found.

In the first 2 patients NGF infusion was associated with a loss of weight and appetite: 6.7 kg in 3 months (12%, patient 1) and 6.5 kg in 3 months (15%, patient 2). This was particularly evident in patient 2, where the weight loss and lack of appetite led to a termination of the NGF infusion after 3 months. In the third patient there was a clear reduction in weight over time (8%), but there was no consistent correlation with the treatment periods and no marked weight gain after the treatment periods (fig. 4).

At the end of the NGF treatment in patients 1 and 2, a few herpes zoster vesicles appeared. Treatment with aciclovir made the vesicles disappear within a few days and no subsequent discomfort was noted. Patients 1 and 2 suffered after 2 and 1 months of NGF treatment, respectively, from sleeping difficulties, anxiety and intermittent periods of confusion. Neuroleptics, benzodiazepines and clomethiazole were given, and the symptoms were alleviated.

Discussion

We report here the treatment of 3 patients suffering from Alzheimer's disease with intracerebroventricular infusion of NGF resulting in both positive and negative results which will be discussed below.

There was no obvious improvement in cognitive performance. The MMSE scores were reduced during treatment. Patients 1 and 2 showed a slight improvement in a few subtests of episodic memory during treatment. Stored information of words and faces could be slightly better remembered, while tests of semantic memory did not improve. This may be indicative of a more effective consolidation of memory traces due to an enhanced function of the cholinergic system. However, the improvements were marginal, not maintained after treatment had been finished and the third patient did not show any such improvements. The third patient on the other hand received the lowest doses of NGF. There was also a considerable variability in the pretreatment results from the cognitive test battery. This patient category is vulnerable to external stimuli and easily distracted. When the infusions started the negative side effects, mainly pain, were

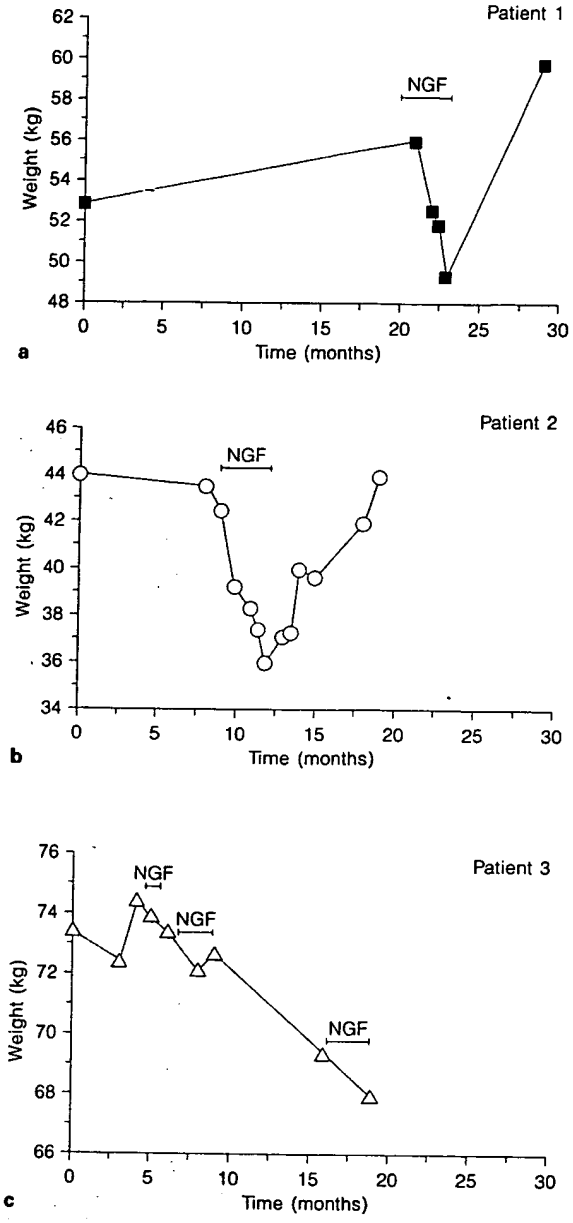

Fig. 4. a, b A marked loss of weight could be observed during treatment in patients 1 and 2 with a clear weight gain upon termination of NGF administration. **c** In patient 3, there is a loss of weight over time (8%), but there is no relation to the NGF treatment periods. There is no weight gain between the treatment periods.

an additional stress factor, causing fatigue and concentration difficulties. The subjects were moderately demented when entering the study yielding low test results already before the start of the infusion, making small improvements difficult to detect. The side effects hindered a long-term follow-up, thus possible long-term effects on cognitive functions remain unknown. Patients 1 and 2 were tested at 3 months after infusion and both showed a decline in most tests, compatible with the natural course of the disease.

A significant loss of nicotinic receptors has been observed in Alzheimer patients both in vitro in studies of postmortem brain tissue [31] and in vivo as studied by PET [28, 29, 32]. In the present study, the binding of ^{11}C-nicotine was expressed as the rate konstant k_2*, which minimizes the influence of cerebral blood flow on the quantitative assessment of ^{11}C-nicotine binding [29]. A negative correlation between cognition and the k_2* value in the temporal cortex has earlier been observed in patients with Alzheimer's disease [29]. A clearly positive effect after continuous intraventricular NGF infusion was the increase in the ^{11}C-nicotine binding in cortical brain regions of the present patients. Interestingly, the effect on ^{11}C-nicotine binding appeared correlated with the dose of NGF. Thus, a clear decrease in k_2* values (increased nicotine binding) was observed in cortical brain regions of patients 1 and 2 following NGF treatment for 3 months while in patient 3, who received much lower doses of NGF, an improvement in ^{11}C-nicotine binding was only seen in the hippocampus (7 months after the end of 10 weeks of treatment with 3.4 μg NGF/24 h). A significant decrease in the k_2* value of ^{11}C-nicotine (increased nicotine binding) has earlier been observed in cortical brain regions of Alzheimer patients treated with the cholinesterase inhibitor tacrine [33] and can be regarded as a sign of improved cholinergic transmission. The increase in nicotinic receptors can be indirect, via an increased amount of acetylcholine in the synaptic cleft [34], or be caused by direct stimulation of the nicotinic receptors [35]. Treatment of PC12 cells with NGF has been shown to increase the number of nicotinic receptors [36, 37]. The finding here that, using low doses of NGF, only the hippocampus showed an increased nicotine binding may reflect the dense network of NGF-responsive cholinergic nerve terminals and/or a high concentration of NGF receptors on the cholinergic terminals in this particular area [38]. The decrease in nicotine binding in the third patient after receiving the lowest dose of NGF may be due to a total lack of effect of NGF in this dose range and thus the normal progression of the disease was monitored.

In the first 2 patients EEG recordings showed a reduction of slow-wave activity during treatment and a simultaneous increase in higher frequencies, approximating a more normal EEG pattern. In elderly subjects, a slowing of the alpha frequency has been shown [39]. Increase in delta activity with age has been correlated with impairment of memory function and with reduced acetylcholinesterase activity in CSF [40]. EEG recordings from patients with Alzheimer's disease show a high percentage of abnormalities [39, 41, 42]. In a longitudinal study of Alzheimer patients, relative delta and theta power increased and alpha and beta power decreased [43]. The 3 patients in our study showed abnormalities in the EEG expected for patients suffering from Alzheimer's disease, especially a slowing of alpha wave activity. The NGF treatment reduced the theta activity in the first 2 patients, who received the higher doses of NGF, leading to a more normal EEG pattern, while the lower doses of NGF in the third patient did not influence the EEG activity.

There are several ways in which NGF delivery might exert beneficial effects in patients with Alzheimer's disease. First, it may arrest the ongoing degeneration of cholinergic projections to the cortices. Second, as indicated by the nicotine binding studies, NGF may cause sprouting of new cholinergic axon terminals in gray matter. Third, there is evidence from animal studies [44] that NGF may have more rapid effects, increasing the firing rates of cholinergic neurons. Recent studies of behavior of rats given intraventricular NGF injections have demonstrated acute behavioral activation, presumed to be mediated by cholinergic activation of nicotinic(?) receptors on dopamine neurons [45, 46]. Finally, there is also some evidence that NGF may exert effects on populations of noncholinergic neurons in the CNS, such as certain GABA neurons [47].

This is the first report showing that NGF infused into the cerebral lateral ventricle in man can induce pain. The 3 patients in this study developed back pain with a relatively sudden onset and of high intensity during treatment. After ending the NGF infusion the pain completely disappeared within a few days but reappeared shortly after resuming the infusion. There was also a clear dose-response relation since the intensity of the pain was related to the concentration of NGF given. In the third patient, the pain was aggravated by axial loading on the spine with shooting pain components of short duration. In this patient the NGF appeared to evoke a latent pain sensation from which he had intermittently suffered for many years prior to the present clinical trial of NGF. In all 3 patients, the pain was typically located to the muscles and soft tissue but no tenderness to pressure was reported. The pain had the characteristics of a nociceptive pain and there were no indications of coexisting neurogenic pain components. There is now increasing evidence that NGF interacts with peripheral pain signalling systems both in animals and man [48, 49]. In the adult animal NGF appears to have selective effects on sensory neurons with nociceptive functions [50]. The generation of pain in our patients may involve both peripheral and central mechanisms. The pain was alleviated using dextropropoxyphene, a drug known to have a central action, while the peripherally acting drugs like salicylic acid, paracetamol and diclofenac were less effective, giving further support to the notion that NGF may cause pain partly by central mechanisms. It has been reported that the excitatory effect of NGF administered in vitro to dorsal root ganglion (DRG) cells may be suppressed by opioid antagonists [51]. It is noteworthy that no pain sensations were noted when considerable amounts of NGF were infused directly into the putamen in 3 patients with Parkinson's disease in order to stimulate intraputaminal adrenal medullary autografts [2, 3]. However, it is not known whether the sensitivity to pain in Alzheimer patients differs from that in healthy subjects. The pain in our patients had similarities to the myalgia experienced following intravenous administration of NGF in healthy subjects [48]. In that study, however, the pain appeared with a latency of only 60–90 min after the injection, whereas in our patients pain was not reported until days after the beginning of the intraventricular infusion. It is known that the time of CSF transport from the cerebral ventricles to the proximal part of the spinal canal is about 1 h and to reach the lowest part of the spinal subarachnoid space 3–6 h are required [10]. Therefore the time required to reach an equilibrium of the NGF concentration in the CSF cannot significantly contribute to the long pain latency observed in our patients. There is no direct access from the subarachnoid CSF space to the DRG, but it is likely that transport of NGF within the root sleeves and diffusion in the epineurial tissue to reach the DRG is a relatively time-consuming process. This may account for the markedly longer pain latencies observed in our patients as compared to the shorter pain latencies in subjects receiving NGF via an intravenous injection. Also in animal studies, a long latency to develop hyperalgesia has been reported. A late mechanical hyperalgesia 7 h after an intraperitoneal injection of NGF in rats has been demonstrated [49]. This may be due to a proliferation of central afferents in the dorsal horn, e.g. substance-P-immunoreactive fibers, maybe leading to the establishment of new synaptic connections [48, 49].

We could not demonstrate any changes in patient substance P concentration in lumbar CSF concomitant with the NGF administration. However, the concentration of neuroactive substances in the CSF poorly reflects changes of their extracellular release within the spinal cord and the amount transported intra-axonally.

At the end of the NGF treatment in patients 1 and 2, a few herpes zoster vesicles appeared, were quickly treated and gave no overt symptoms. It is general clinical knowledge that herpes zoster lesions may be preceded by 2–3 days by pain within the dermatome. The pain observed in our patients, however, developed within a few days after the start of the NGF treatment, about 3 months before the appearance of the herpes zoster vesicles, and disappeared when the NGF infusion ended. Moreover, there were no neurogenic pain components in the 3 patients. Thus, we do not think that the herpes zoster infection is of any relevance for the development of pain in the patients.

Weight loss has been noted in experimental animals following chronic NGF infusion [52]. It is also known that Alzheimer patients lose weight as the disease itself progresses. The diagnosis of Alzheimer's disease is a significant predictor of a weight loss of approximately 5% per year [53]. However, in patients 1 and 2, a rapid weight loss of 12–15% over 3 months followed by a quick weight gain after infusion was seen. The mechanisms remain to be elucidated, but may possibly involve effects of NGF in hypothalamic centers. The weight of patient 3 showed a steady decline irrespective of NGF treatment. His weight loss was similar to that seen in other Alzheimer patients.

There is a marked difference between intracerebral and intracerebroventricular delivery of NGF. Intraputaminal administration of NGF continuously for 4 weeks (a total of 3.6 mg) to parkinsonian patients did not cause any noticeable side effects [2, 3]. Patient 3, who was treated with markedly lower doses of NGF than patients 1 and 2, did not show any improvements in neuropsychology testing, nor in EEG activity, but an increase in nicotinic binding, albeit limited to the hippocampal area. The amount of NGF given intraventricularly was probably too low to stimulate NGF receptors in most other areas. In rats it has been shown that the intracerebroventricular dose of NGF must be approximately 100 times higher than the intraparenchymal dose to equally activate the NGF receptors [54]. The consistent appearance of pain and weight loss in our patients treated with NGF with the obvious dose-dependent relationship no doubt presents an obstacle to the possible treatment of degenerative disorders with intraventricular administration of NGF. On the other hand, intraventricular NGF does lead to positive trophic effects including upregulation of nicotinic receptors in the brain, an increased cortical blood flow, a decrease in slow-wave EEG activity and a slightly improved performance in certain cognitive tests. It is therefore important to attempt minimizing adverse side effects while maintaining and strengthening the positive trophic effects of NGF. One way to minimize the undesirable side effects when using NGF and other trophic factors may be the use of intraparenchymal application.

To conclude, the several different indications of positive effects noted in this limited pilot study suggest that activation of the NGF receptor system (p75 and TrkA) [55] may be beneficial in Alzheimer's disease. Alternatives to delivery of exogenous protein include implantation of cells genetically modified to secrete NGF, gene transfer, low-molecular-weight molecules with neurotrophic effects (immunophilins) [56] as well as ways to stimulate endogenous NGF synthesis. Further preclinical research to elucidate the mechanisms behind the NGF-induced pain and weight loss is also needed.

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